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Guido Vitale, Laure Laforgue, Richard Michel, Jocelyn Etienne, Valérie M. Laurent, Alain Duperray, Claude Verdier

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G. Vitale[1,2], L. Laforgue[1,2], R. Michel[1,2], J. Étienne[1,2], V. M. Laurent[1,2], A. Duperray[3,4,5], C. Verdier[1,2].

[1] *Univ. Grenoble Alpes, LIPHY, F-38000, Grenoble, France.*
[2] *CNRS, LIPHY, F-38000, Grenoble, France.*
[3] *INSERM, IAB, F-38000, Grenoble, France.*
[4] *Univ. Grenoble Alpes, IAB, F-38000, Grenoble, France.*
[5] *CHU de Grenoble, IAB, F-38000, Grenoble, France.*

E-mail: guido.vitale@ujf-grenoble.fr, vitale.gui@gmail.com

Abstract: Traction Force Microscopy (TFM) is an inverse method that allows to obtain the stress field applied by a living cell on the environment on the basis of a pointwise knowledge of the displacement produced by the cell itself during its migration. This biophysical problem, usually addressed in terms of Green functions, can be alternatively tackled in a variational framework. In such a case, a suitable penalty functional has to be minimized. The resulting Euler-Lagrange equations include both the direct problem based on the linear elasticity operator as well as an equation built on its adjoint. Results from a two-dimensional model, i.e. where living cancer cells are migrating on a plane substrate, are briefly presented. While the mathematics is well established also in the three-dimensional case, i.e. where cells are completely embedded in the gel matrix, the experimental data needed are more difficult to obtain than the two-dimensional counterpart. First steps towards the complete three-dimensional traction reconstruction are reported.

Keywords: Traction Force Microscopy, Inverse Problem, Three-dimensional Cell Migration.

1 Introduction

Many living cells have the ability to migrate, both in physiological and pathological conditions; examples include wound healing, embryonic morphogenesis and the formation of new vessels in tumours. The motility of a cell is driven by the reorganization of its inner structure, the cytoskeleton, according to a complex machinery. The net effect of this process is that a cell is able to apply a stress on the environment, pulling the surrounding material to produce its own movement. Therefore, the determination of forces on the basis of measured displacement, has become a popular problem in the biophysical community.

The early idea to study the force applied by cells in their migration (so called Traction Force Microscopy, TFM) on flat substrate as an inverse problem dates back to the work of Harris and coworkers in the 1980s [6]. Afterwards, Dembo and Wang [4] came up with a new methodology: the living cell is again put on an elastic substrate and the displacement of fluorescent beads dispersed in such material is evaluated from different images. Finally, they solve the problem of elasticity in the substrate in terms of Green functions and then minimize the error between the measured and the calculated displacement under Tichonov regularization.

An alternative approach can be stated in a continuous variational framework [1]. Again, the starting point is a Tichonov functional defined as the displacement error norm plus a regularization. If a variation of such a functional is operated, the definition of an adjoint problem for the unknown force naturally arises. By doing this, two coupled elliptic partial differential equations are obtained and their solution can be approximated, for instance, using finite element.

Although that approach is less common than the seminal method based on Green functions, it has some attractive features that make it worth investigating further: it can be generalized easily to more complex geometry and material behaviour including non-linearities.

In this work we aim to recall the mathematical structure of the TFM problem. We then present some results of the well established two-dimensional theory for a living cancer cell migrating on a plane substrate. Finally, some early data corresponding to an istance of the transmigration of a living cell in a three-dimensional environment are shown.

2 Mathematical Setting

The Direct Problem of Linear Elasticity

The stationary force balance equations on a linear elastic continuum body $\Omega \subset \mathbb{R}^3$ writes:

$$\begin{cases} -\mathbf{div}\,(\mathbb{A}\nabla \boldsymbol{u}) & = 0, \qquad \text{in } \Omega, \\ (\mathbb{A}\nabla \boldsymbol{u})\,\boldsymbol{n} & = \boldsymbol{T}, \qquad \text{on } \Gamma_{\mathrm{N}}, \\ \boldsymbol{u} & = 0, \qquad \text{on } \Gamma_{\mathrm{D}}. \end{cases} \tag{1}$$

where $\boldsymbol{u}$ is the displacement field. The domain Ω represents the gel. The boundaries $\Gamma_{\mathrm{D}}, \Gamma_{\mathrm{N}} \subset \partial\Omega$ are such that $\Gamma_{\mathrm{N}} \cap \Gamma_{\mathrm{D}} = \varnothing$, $\overline{\Gamma_{\mathrm{N}} \cup \Gamma_{\mathrm{D}}} = \partial\Omega$ and $\boldsymbol{n}$ is the unit outward normal of the boundary $\partial\Omega$. On the Neumann boundary Γ_{N} a load is applied per unit surface (here referred as 'traction' and denoted with $\boldsymbol{T}$) is applied. The region where the cell and the gel are in contact, say Γ_{c}, is a subset of Γ_{N}. The set Γ_{c} is indeed the support of the traction field $\boldsymbol{T}$. Last, $\mathbb{A}$ is the fourth order Hooke elasticity positive tensor.

For notational convenience, we define the solution operator $\mathcal{S}$, as the map that, for a given control $\boldsymbol{T}$, assigns the displacement field $\boldsymbol{u}$ that solves the elasticity problem:

$$\boldsymbol{u} = \mathcal{S}\boldsymbol{T} \quad \text{ if and only if } \quad \boldsymbol{u} \text{ solves (1)}.$$

Two–dimensional approximations of the elasticity equation have already been succesfully employed in the field of TFM [1]. They rely on the existence of suitable averaging operators [8] whose action is denoted by an overbar ($\bar{}$). Let us consider Ω being a cylinder with Γ_{N} the upper surface, i.e. the plane of cell migration. In [1] it is shown that the system (1) can be averaged in the direction orthogonal to Γ_{N} and that gives:

$$\begin{cases} -\mathbf{div}\,\left(\bar{\mathbb{A}}\nabla \bar{\boldsymbol{u}}\right) & = \boldsymbol{T}, \qquad \text{in } \Gamma_{\mathrm{N}}, \\ \bar{\boldsymbol{u}} & = 0, \qquad \text{on } \partial\Gamma_{\mathrm{N}}. \end{cases} \tag{2}$$

where $\bar{\mathbb{A}}$ denotes the depth-averaged elasticity tensor. Observe that on the Γ_{N} boundary, Dirichlet conditions apply.

Available Experimental Data

Standard imaging techniques are avaible in order to detect the cell, thus giving the region $\Gamma_{\mathrm{c}} \subseteq \Gamma_{\mathrm{N}}$.

The experimental devices are able to give information on the actual gel displacement field. The position of small fluorescent beads embedded into the gel can be, indeed, tracked for the case of a plane substrate. When dealing with a three-dimensional environment, the position of the intersection of the gel fibers can be similarly obtained. This latter situation represents a novelty from the point of view of data acquisition in TFM and it is currently under development. In view of the smallness of both the beads and the fiber intersection area, we are basically dealing with pointwise measurements of the displacement field. That remark justifies the representation of the observation operator as a list of Dirac mass centered at bead positions. Then, the observation operator writes $\mathcal{O} := (\delta_{\boldsymbol{x}_1}, \ldots, \delta_{\boldsymbol{x}_N})$, where $\boldsymbol{x}_k$ denotes the position of the bead labeled with the number k and $\delta_{\boldsymbol{x}_k}$ is the Dirac mass located at $\boldsymbol{x}_k$.

Biomechanical Constraints

When dealing with applications, the need to include constraints into the model appears. It seems that this was not explicitly stated in the previous literature on TFM, except [10, 11, 8]. We note, indeed, that

- since some reasonably accurate images of the cell are avalaible, the support of the traction field $\boldsymbol{T}$ can be determined, leading to the constraint $\operatorname{supp} \boldsymbol{T} \subseteq \Gamma_c$;
- the mechanical balance of the cell requires $\int_{\Gamma_c} \boldsymbol{T} = 0$ and $\int_{\Gamma_c} \boldsymbol{x} \times \boldsymbol{T} = 0$;

In the following, the space of tractions satisfying the above constraints will be called admissible and denoted with

$$\mathsf{F}_{\text{adm}} := \{\boldsymbol{T} : \Gamma_N \to \mathbb{R}^d \mid \int_{\Gamma_c} \boldsymbol{T} = 0,\ \int_{\Gamma_c} \boldsymbol{x} \times \boldsymbol{T} = 0,\ \boldsymbol{T} = 0 \text{ a.e. on } \Gamma_N \setminus \Gamma_c\}. \tag{3}$$

Where $d = 2$ or 3 depending on the specific application. Note that F_{adm} is a linear subspace of the space of tractions [10]. The projection on the space F_{adm} is denoted by P.

The Inverse Problem

The information provided experimentally to solve the inverse problem, i.e. the pointwise measurements of the displacement $\boldsymbol{u}$, are not sufficient to yield a unique traction field $\boldsymbol{T}$. The problem is therefore underdetermined and, as customary, we have to enforce a suitable minimization problem to circumvent this drawback.

Tichonov Functional

The Tichonov functional is defined as:

$$\mathcal{J}(\boldsymbol{T}) = \frac{1}{2}\|\mathcal{O}\mathcal{S}\boldsymbol{T} - \mathbf{u_0}\|^2 + \frac{\varepsilon}{2}\|\boldsymbol{T}\|^2. \tag{4}$$

Here $\mathbf{u_0} = (\boldsymbol{u}_0^1, \ldots, \boldsymbol{u}_0^N)$ are the known displacements at $\boldsymbol{x}_1, \ldots, \boldsymbol{x}_N$ respectively. The Tichonov functional $\mathcal{J}$ is the sum of two parts: the first term is the discrepancy between the measured displacement $\mathbf{u_0}$ and the calculated displacement for a given force $\boldsymbol{T}$ (i.e. $\mathcal{S}\boldsymbol{T}$), evaluated at the beads location (i.e $\mathcal{O}\mathcal{S}\boldsymbol{T}$); the second one is the force magnitude. The two additive contributions are weighted by the positive constant ε, the regularization parameter.

This traction field $\boldsymbol{T}$ is thus defined as the (unique) minimizer of the Tichonov functional $\mathcal{J}$ in the set of the admissible tractions F_{adm}. It therefore satisfies:

$$P\mathcal{J}'(\boldsymbol{T}) = \varepsilon\boldsymbol{T} + P(\mathcal{O}\mathcal{S})^T(\mathcal{O}\mathcal{S}\boldsymbol{T} - \mathbf{u_0}) = 0. \tag{5}$$

Adjoint Equation

Although the stationarity condition (5) is in principle sufficient to define the optimal $\boldsymbol{T}$, it is convenient to reformulate the problem in terms of a differential equation. It turns useful to define the adjoint state $\boldsymbol{p}$ as

$$\mathcal{S}^{-T}\boldsymbol{p} = \mathcal{O}^T(\mathcal{O}\boldsymbol{u} - \mathbf{u_0}), \tag{6}$$

where $\mathcal{S}$ is the solution operator and $\boldsymbol{u}$ is the displacement field, as defined in equation (1). Substituting (6) into (5), we find the relationship between the optimal force $\boldsymbol{T}$ and the adjoint state:

$$\varepsilon\boldsymbol{T} + P\boldsymbol{p} = 0 \tag{7}$$

The presence of the projection P requires, when solving the problem in practice, the introduction of Lagrange multipliers as done in [10, 8]. Since it can be shown that $\mathcal{S}$ is self adjoint [3], equation (6) can be rewritten in a more familiar way as follows:

$$\begin{cases} -\mathbf{div}(\mathbb{A}\nabla\boldsymbol{p}) & = \mathcal{O}^T(\mathcal{O}\boldsymbol{u} - u_0), & \text{in } \Omega, \\ (\mathbb{A}\nabla\boldsymbol{p})\boldsymbol{n} & = 0, & \text{on } \Gamma_N, \\ \boldsymbol{u} & = 0, & \text{on } \Gamma_D. \end{cases} \tag{8}$$

The latter equation is specific for the three dimensional problem. It is easy to deduce the adjoint equation relative to the two-dimensional averaged case (2), which can be found in [1, 8].

Numerical Approximation

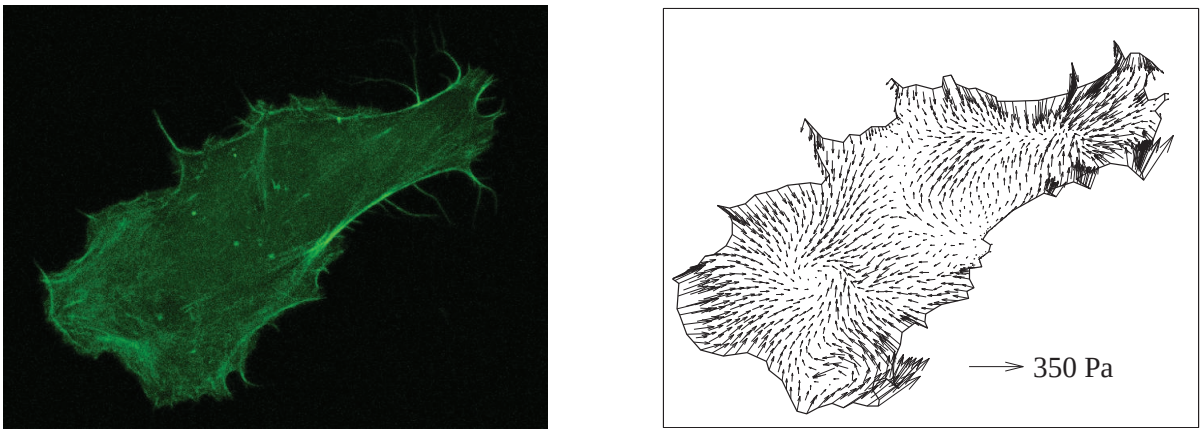
Summarizing, our differential problem in strong form is represented by the system of equations (1) and (8) supplemented by the definition of the adjoint state (7) and the constraints (3). Well–posedness of the aforementioned system and further details are avaible in [10]. The numerical implementation using Finite Elements is thus straightforward. The choice of the regularization parameter ε is made by the use of the L-curve method [5]. A preliminary validation test in the three-dimensional case can be seen in [11].

Our approach differs from [7] where the three dimensional TFM problem is tackled using numerically approximated Green functions. However, no details are provided on the mathematical well–posedness. Also, the latter method does not permit to incorporate the biomechanical constraints mentioned here. In addition, the Green function-based method is proved to be computationally more expensive as compared to ours [11].

3 Applications

The well established tool for evaluating traction forces on a plane substrate [1] has been succesfully applied when studying the migration of a living cancer cell [2]. In Fig. 1a) a living cancer cell is shown while migrating on a plane substrate and is tagged with fluorescence. Fig. 1b) shows the corresponding traction map. Relevant biophysical facts that may be deduced from two-dimensional TFM are detailed in [9].

The more difficult case corresponding to migration in a three-dimensional environment is currently under study. An image of the cell embedded in a collagen matrix is shown in Fig. 2a). In this case, the displacement field is measured using an auto-correlation technique and can be reconstructed pointwise. An interpolation has been required in order to visualize the latter as a colormap on a section plane in Fig.2b), whereas it is not necessary for the numerical calculations.



(a) (b)

Figure 1. *a) GFP actin fluorescent living cancer cell during migration on a plane substrate; b) Traction map.*

The force reconstruction is, therefore, possible since all the ingredients are available. Together with a discussion of the biological implications of such data, that will be the subject of forthcoming work by our lab.

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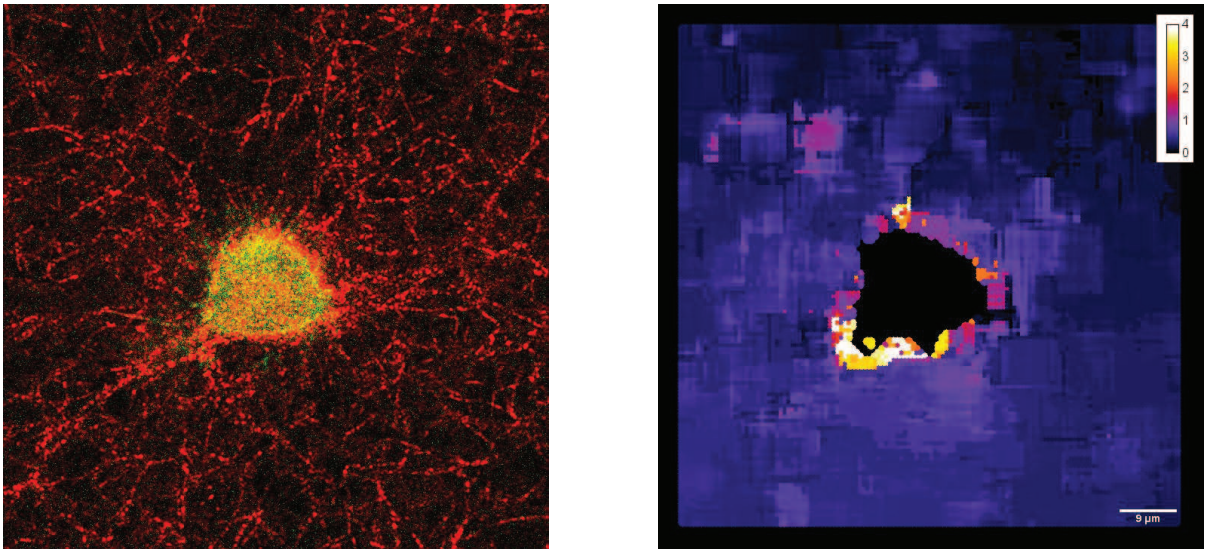

(a) (b)

Figure 2. *a) A living cancer cell during its migration in a three dimensional collagen gel (confocal microscopy, z-slices); b) colormap of the magnitude of the displacement field (µm) induced by the cell in the z-plane (interpolated on the whole domain just for visualization).*

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